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EFFECT OF EXPERIMENTAL CHOLESTASIS ON NEUROMUSCULAR BLOCKING DRUGS IN CATS

P. WESTRA, M. C. HOUWERTJES, A. R. DE LANGE, A. H. J. SCAF, F. R. HINDRIKS AND S. AGOSTON

SUMMARY

Pancuronium, Org 6368 and gallamine were compared in control cats and in cats with experimental cholestasis. A decrease in the plasma clearance and a prolongation of neuromuscular blockade with Org 6368 and pancuronium were found in the latter; no significant difference was detected in the biotransformation pattern of Org 6368 and pancuronium compared with controls. Inhibition of hepatic uptake of Org 6368 and pancuronium in extrahepatic cholestasis might explain the significant alterations in the pharmacokinetics of the two steroid neuromuscular blocking drugs. The pharmacokinetics of gallamine were normal during cholestasis. The results suggest that, under pathological conditions involving increased plasma concentrations of bile salts, neuromuscular blocking agents that are cleared from the plasma by the liver may have an impaired hepatic uptake and consequently a prolonged duration of action.

Cholestasis is associated with a variety of metabolic abnormalities some of which, for example jaundice and increased plasma bile salt concentrations with steatorrhea, are the direct result of the failure of bilirubin and bile salt secretion into bile. However, certain other phenomena associated with extrahepatic cholestasis are more difficult to explain. Examples are the increased duration of action of hexobarbitone in rabbits with obstructive jaundice (McLuen and Fouts, 1961) and a prolongation of the sleeping time caused by the steroid anaesthetic Althesin (Novelli, Marsili and Lorenzi, 1975). Ward, Adu-Gyamfi and Strunin (1975) reported difficulties in antagonizing the effects of pancuronium in two patients with severe obstructive jaundice. Significant changes in the elimination of pancuronium which resulted in a prolonged neuromuscular blockade in patients with total biliary obstruction, were reported by Somogyi, Shanks and Triggs (1977).

The liver takes up many drugs from the bloodstream and plays an important role in the metabolism and excretion of these compounds. Interference with

either of these processes will affect the pharmacokinetic pattern of such drugs. This may have clinical consequences such as alterations in their duration of action.

In the case of the neuromuscular blocking drugs tubocurarine (Cohen, Brewer and Smith, 1967; Meijer and Scaf, 1968; Meijer et al., 1979), pancuronium (Agoston et al., 1973; Buzello, 1975) and hexafluorenum (Meijer, Vermeer and Kwant, 1971), hepatic uptake and secretion appear to be major factors in determining the plasma concentrations. In the cat, hepatic uptake is an important factor in the plasma clearance of a number of steroid neuromuscular blocking agents, such as pancuronium, dacturionium and Org 6368 (Agoston, Kersten and Meijer, 1973; Agoston et al., 1977).

We investigated the fate of non-depolarizing neuromuscular blocking agents in extrahepatic cholestasis. Three compounds, known to differ considerably in their elimination pathways (i.e. renal or biliary), were studied:

Org 6368, the bisquaternary ammonium compound (2 β ,16 β -dipiperidino-5 α -androstan-3 α -ol acetate dimethobromide) differing from pancuronium only in lacking the acetate group at C17 position in the steroid skeleton and having a short duration of action in the cat (Sugrue and Duff, 1973), mainly as a result of a rapid hepatic uptake (Agoston et al., 1977); *gallamine triethiodide*, which in dog and man is predominantly eliminated by the kidneys (Mushin et al., 1949; Feldman, Cohen and Golling, 1969; Agoston et al., 1978);

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pancuronium, which in cat and man is eliminated by both urinary and biliary routes (Agoston, Kersten and Meijer, 1973; Agoston et al., 1973).

The dynamics and kinetics of these compounds were investigated in cats with experimentally induced cholestasis and compared with control animals.

MATERIALS AND METHODS

Operative procedures

Ligation of the bile duct was performed under anaesthesia with sodium pentobarbitone anaesthesia (40 mg kg^{-1} i.p.), or 2% halothane in 33% oxygen in nitrous oxide after ketamine 50–100 mg i.m. An endotracheal tube was inserted and the lungs ventilated artificially. A mid-line abdominal incision was made, and the common bile duct was doubly ligated with Perma-Hand Seide 4-0 suture approximately 1 cm from the point of entry to the duodenum. The cystic duct was doubly ligated at the base of the gall-bladder, after which the incision was closed with Perma-Hand Seide. The entire operation took less than 30 min. After the operation, all animals were treated with ampicillin 100–200 mg, i.m. The obstruction of the bile ducts lasted 9–10 days. Sham-operated (all manipulations without ligation) and un-manipulated cats served as controls.

Animal experiments

All experiments were carried out in adult cats ($2.8 \pm 0.2 \text{ kg}$) of either sex under pentobarbitone anaesthesia (40 mg kg^{-1} , i.p. plus maintenance doses of 4 mg kg^{-1} i.v.). After orotracheal intubation, artificial ventilation with air through a cuffed Magill tube of 5.5 mm external diameter was maintained with a Braun respirator type 1955, set at a rate of 30 b.p.m. and a tidal volume of 10 ml kg^{-1} . A polythene cannula was introduced into the common bile duct and in the control animals the cystic duct was ligated. Through a low abdominal incision, the urethra and the urinary bladder were visualized and the urethra was cannulated. In order to check the general condition of the cat, e.c.g. (ECG-Pulse Monitor, MS-35; Electrodyne Co. Inc., U.S.A.) and mean arterial pressure (Statham P23D6-transducer; KWS 3085 HSE Electro-Manometer) were monitored. Body temperature was kept constant at $36\text{--}38^\circ\text{C}$ by heating the operating table. Rectal temperature was measured with a thermistor (Electrolaboriet, Copenhagen). To replace fluid loss, glucose (2.5%) and saline (0.45%) were infused through a polythene cannula in the external jugular vein. Neuromuscular studies (isometric twitch tension)

were performed on the tibialis anterior muscle which was stimulated through the common peroneal nerve with supramaximal square-wave pulses of 0.2-ms duration at a frequency of 0.1 Hz. The stimulus was supplied from a Grass S88 stimulator and SIU 5 stimulus isolation unit. The tendon of the tibialis anterior muscle was connected to a load cell (UL4-20, Statham), attached to a force-displacement transducer (UC3 Gold Cell, Statham), mounted securely on a Braun-Schuster myograph. The signal was amplified by a Hottinger-Baldwin amplifier (Messtechnik, Darmstadt) and muscle contractions were continuously recorded on a MFE two-channel polygraph (d.c. to 40 Hz, full scale). Pancuronium 0.3 mg kg^{-1} , Org 6368 1.5 mg kg^{-1} and gallamine 5.0 mg kg^{-1} were injected i.v. over 5 s. Blood, urine and bile samples were collected at fixed intervals. Eight hours after administration of the drugs, the experiments were terminated by removing the liver within 2 min. All samples were deep frozen for later chemical analysis.

Neuromuscular studies

The time to the onset of maximum block was defined as the time which elapsed from the injection until the twitch had reached its minimum value. For the duration of action, two indices were used:

- time from administration of the drug until the return of the twitch tension to 50% of control value (duration 50);
 - time from administration of the drug until reversal to 90% of control tension (duration 90).
- Recovery time is defined as the time from the minimum value before recovery to 25, 50 and 75% recovery of control twitch tension (recovery 25, 50, 75). The recovery rate is the time from 25 to 75% recovery of control twitch tension (recovery rate 25–75) (fig. 1).

Analysis of the quaternary ammonium compounds and of some physiological plasma constituents

Determinations of plasma concentrations of total bilirubin, direct bilirubin, total cholesterol, glutamic-pyruvic transaminase, alkaline phosphatase, urea and creatinine of the cats were performed with an Automatic Chemical Analyzer of E. I. du Pont de Nemours & Co. (Inc.), Wilmington, DE 19898. Enzyme activities are expressed in international units per litre (iu litre^{-1}).

Total bile salt concentrations in the plasma were measured with an enzymatic kit for spectrofluorimetric end-point determinations (Sterognost-3a Automated, Nyegaard & Co. AS, Oslo, Norway).

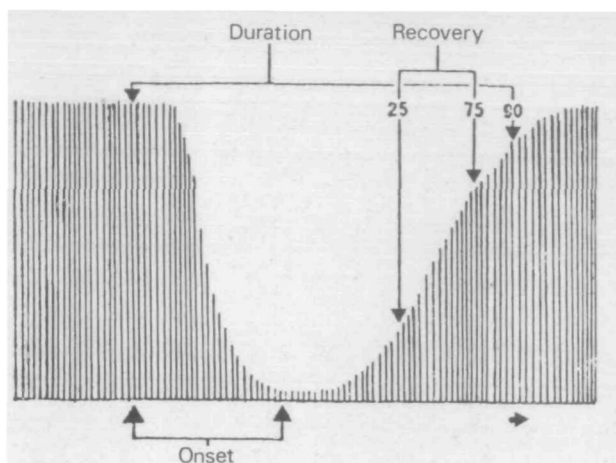


FIG. 1. Characterization of the neuromuscular blockade.

3 α -Hydroxysteroid dehydrogenase transforms the 3 α -hydroxyl group on steroids of the C19, C21 and C24 series into their corresponding keto forms, by which NADH is generated from NAD (Mashige, Imai and Osuga, 1976; Osuga et al., 1977). The reduction equivalents of NADH are transferred to the fluorogen resazurin by the enzyme diaphorase, with formation of the fluorophore resorufin. This substance is measured with an Aminco-Bowman Spectrofluorimeter (Am. Instr. Comp.). Excitation wave length was 565 nm and emission wave length 580 nm.

Spectrofluorimetric determinations of pancuronium Org 6368 and gallamine and thin-layer chromatography of these bis(tris)-quaternary ammonium compounds and metabolites have been described in detail elsewhere (Kersten, Meijer and Agoston, 1973; Agoston et al., 1977; Agoston et al., 1978).

The fluorimetric method for bisquaternary ammonium compounds does not discriminate between pancuronium, Org 6368 and their deacetylated metabolites(s). Chromatographic quantification of the bisquaternary ammonium compounds in bile and liver homogenate as described previously (Kersten, Meijer and Agoston, 1973; Agoston et al., 1977) was improved by mixing the samples (1 ml) with 0.1 ml of a saturated copper sulphate solution and removing the precipitated material by centrifugation. The supernatant was used for further chromatographic procedures.

Conjugation

To investigate a possible conjugation of Org 6368 or metabolite(s) with sulphate or glucuronic acid

liver homogenates of control cats receiving Org 6368 1.5 mg kg⁻¹ were analysed.

Sulphate conjugation: to 2 ml of liver homogenate (1 mg of tissue in 1 ml Krebs-bicarbonate) 1 ml of Tris-buffer 2 mol litre⁻¹ (pH = 7.4) and 100 μ litre partially purified bacterial aryl sulphatase solution (Sigma, St Louis, U.S.A.) were added.

Glucuronidation: to 2 ml of liver homogenate, 1 ml of sodium acetate-buffer 0.5 mol litre⁻¹ (pH = 6.0) and 100 μ litre of highly purified bacterial glucuronidase solution (Boehringer, Mannheim, West Germany) were added. The mixtures were adjusted to 15 ml with distilled water and incubated for 2.5 h at 37 °C. The same liver homogenates without addition of the enzyme preparations served as controls. Fluorescence of non-enzyme treated and enzyme-treated livers containing Org 6368 or its metabolite(s) were compared. This method is based on the assumption that the polar conjugates of the metabolite(s) of Org 6368 will not be extracted with Rose-Bengal into the chloroform phase (Buzello, 1975).

Statistics

All values are given as mean \pm SEM. Statistical significance was tested using either the paired or the unpaired Student's *t* test with a 95% significance level.

Pharmacokinetic calculations

The disposition of neuromuscular blocking drugs can be described mathematically by a two- or three-compartment open model (Gibaldi, Levy and Hayton, 1972; McLeod, Watson and Rawlins, 1975; Somogyi, Shanks and Triggs, 1976; Miller et al., 1979).

Plasma clearance curves were fitted using a computerized program which, after balanced iterative peeling of the curves, yields straight regression lines of log plasma concentration *v.* time, using the least squares method. Pharmacokinetic model-parameters, such as steady-state volume of distribution (V^{∞}), volume of central compartment (V_1) and plasma clearance (*Cl*) were calculated according to Wagner (1976).

RESULTS

Clinical chemistry

There was no impairment of renal glomerular filtration in the cats with extrahepatic cholestasis, as judged by normal plasma concentrations of creatinine and urea. The liver function tests revealed

significant increases of the enzymes alkaline phosphatase (AP) and glutamic-pyruvic transaminase (GPT) in the jaundiced cats. Significant increases in the plasma concentrations of total cholesterol and total bilirubin were observed. Approximately 60% of the total bilirubin in the plasma was present as a glucuronide conjugate.

Total bile salt concentrations in plasma, after 9–10 days experimental cholestasis, were increased more than 100-fold as compared with control. Relief of biliary obstruction by cannulation of the common bile duct caused a linear decay of the plasma bile salt concentration, indicating saturation elimination kinetics. After 7 h the concentration was about half

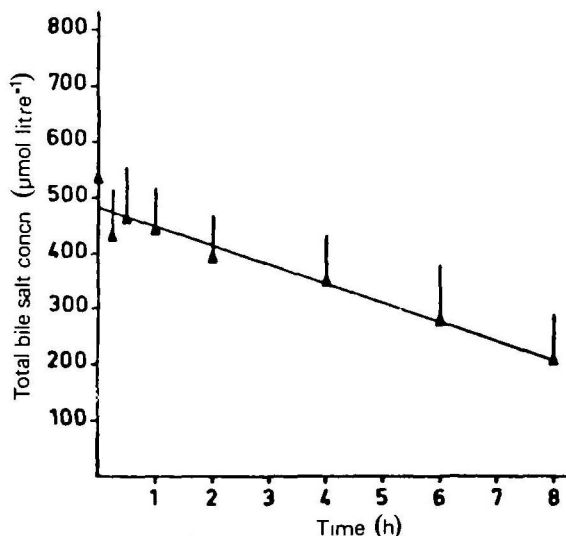


FIG. 2. Total bile salt concentration in plasma after relief of the biliary obstruction ($n = 12$). Mean \pm SEM.

the initial value (fig. 2). Total cholesterol concentrations had returned to baseline after 480 min and total bilirubin plasma concentration was decreased by about 40% at that time (table I).

Pharmacodynamics

The pharmacodynamic indices defining the neuromuscular blockade of Org 6368 in cats with experimental cholestasis differed clearly from those of the control animals, except for the time of onset of action. The same is true, but to a lesser extent, for pancuronium (table II).

Cholestasis produced a 5.5-fold prolongation of the duration to 90% twitch recovery with Org 6368 compared with controls; for pancuronium this prolongation was only 1.6-fold (fig. 3). In contrast,

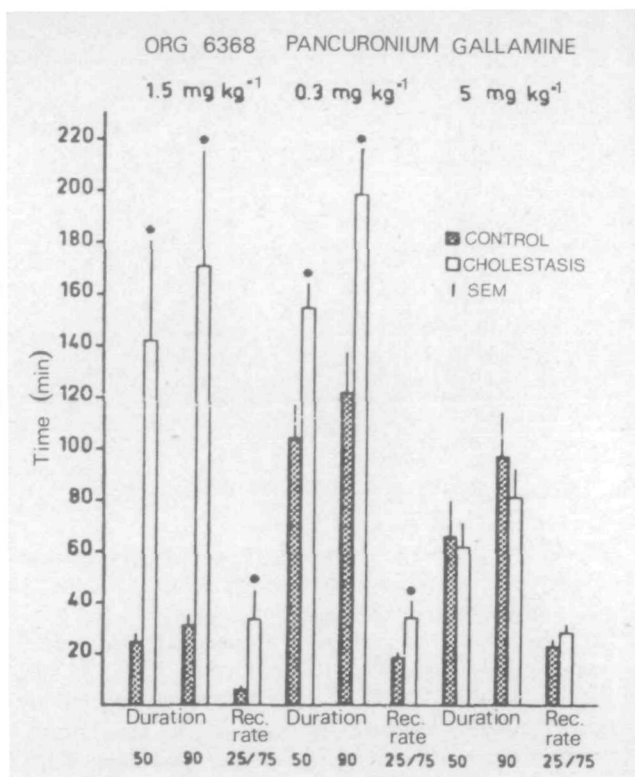


FIG. 3. Duration to 50 and 90% recovery of control twitch tension and the recovery rate from 25 to 75% of control twitch tension for Org 6368, pancuronium and gallamine in control cats and cats with cholestasis. Mean \pm SEM.

* Significant difference from control.

cholestasis had no influence on the dynamics of gallamine (table II). Also, no significant differences between un-manipulated control cats and sham-operated cats were found for Org 6368 in this respect (table II).

Pharmacokinetics of Org 6368

The pharmacokinetic indices such as the half-lives ($T_{1/2}^{\alpha}$, $T_{1/2}^{\beta}$ and $T_{1/2}^{\gamma}$), the plasma clearance (Cl), the volume of central compartment (V_1) and the steady-state volume of distribution (V_{ss}) of the three quaternary ammonium compounds are given in table IV. For Org 6368, a considerable decrease in plasma clearance and significant increases of the distribution half-life $T_{1/2}^{\alpha}$ and of the elimination half-life $T_{1/2}^{\beta}$ were found in extrahepatic cholestasis (table IV, fig. 4).

The cumulative urinary excretion of Org 6368 (fig. 5) in extrahepatic cholestasis revealed a profound increase, from 10% to approximately 40% of the

TABLE I. Analysis of plasma just before relief of the biliary obstruction ("0") and 480 min after administration of the drugs. Mean \pm SEM. Figures in parentheses indicate range. * Significant difference from controls. AP = alkaline phosphatase; GPT = glutamic-pyruvic transaminase

	Total bile salt ($\mu\text{mol litre}^{-1}$)	Total bilirubin ($\mu\text{mol litre}^{-1}$)	Direct bilirubin ($\mu\text{mol litre}^{-1}$)	Total cholesterol (mmol litre $^{-1}$)	AP (iu litre $^{-1}$)	GPT (iu litre $^{-1}$)	Urea (mmol litre $^{-1}$)	Creatinine ($\mu\text{mol litre}^{-1}$)
Control "0" min <i>n</i> = 12	1.7 \pm 0.7 (0-4.1)	4 \pm 1 (2-7)	3 \pm 1 (0-7)	2.18 \pm 0.21 (1.30-2.83)	18 \pm 10 (0-81)	53 \pm 7 (30-102)	8.2 \pm 0.5 (5.9-11.6)	87 \pm 6 (60-105)
Sham "0" min <i>n</i> = 3	0.7 \pm 0.4 (0-1.2)	4 \pm 1 (3-4)	0	2.80 \pm 0.77 (1.55-4.19)	29 \pm 20 (2-63)	56 \pm 18 (23-83)	11.0 \pm 0.2 (10.6-11.4)	88 \pm 12 (70-110)
Cholestasis "0" min <i>n</i> = 13	521 \pm 119* (81-794)	171 \pm 29* (44-343)	105 \pm 18* (30-216)	3.24 \pm 0.26* (1.11-3.90)	103 \pm 29* (51-169)	741 \pm 98* (521-1550)	9.2 \pm 0.9 (4.7-17.6)	88 \pm 7 (80-115)
Cholestasis 480 min <i>n</i> = 9	212 \pm 82* (0-422)	107 \pm 25* (27-248)	62 \pm 13* (14-133)	2.38 \pm 0.29 (1.18-3.80)	—	—	—	—

TABLE II. Quantification of neuromuscular blockade after i.v. administration of Org 6368, pancuronium and gallamine in control cats, sham-operated cats (only in case of Org 6368) and cats with cholestasis. Mean \pm SEM. * Significant difference from controls

	Drug ($\mu\text{g kg}^{-1}$)	Block (%)	<i>n</i>	Onset (min)	Duration (min)		Recovery (min)			Recovery rate (min) 25-75
					50	90	25	50	75	
Control	Org 6368 1500	100	5	0.63 \pm 0.07	24.65 \pm 3.42	30.81 \pm 4.08	4.33 \pm 0.56	6.87 \pm 1.00	10.50 \pm 1.72	6.10 \pm 1.17
Sham	Org 6368 1500	100	3	0.56 \pm 0.06	18.80 \pm 2.39	24.22 \pm 3.47	4.05 \pm 0.62	6.06 \pm 1.03	8.50 \pm 1.69	4.50 \pm 1.07
Cholestasis	Org 6368 1500	100	5	0.56 \pm 0.04	142.20* \pm 38.64	170.72* \pm 44.97	18.90* \pm 5.08	35.50* \pm 9.51	50.17* \pm 25.08	33.38* \pm 11.26
Control	Pancuronium 300	100	5	0.77 \pm 0.04	104.10 \pm 13.26	121.83 \pm 15.78	14.64 \pm 1.77	23.77 \pm 3.08	33.17 \pm 3.95	18.53 \pm 2.59
Cholestasis	Pancuronium 300	100	4	0.75 \pm 0.05	154.71* \pm 10.34	199.13* \pm 17.89	26.59* \pm 3.09	39.81* \pm 4.31	60.87* \pm 5.95	34.28* \pm 7.04
Control	Gallamine 5000	100	5	0.77 \pm 0.07	66.25 \pm 13.39	96.99 \pm 18.28	16.70 \pm 2.15	27.58 \pm 3.94	39.87 \pm 4.62	23.17 \pm 3.09
Cholestasis	Gallamine 5000	100	5	0.75 \pm 0.05	61.68 \pm 11.53	81.42 \pm 11.32	18.05 \pm 2.68	31.73 \pm 3.94	46.37 \pm 5.51	28.31 \pm 3.51

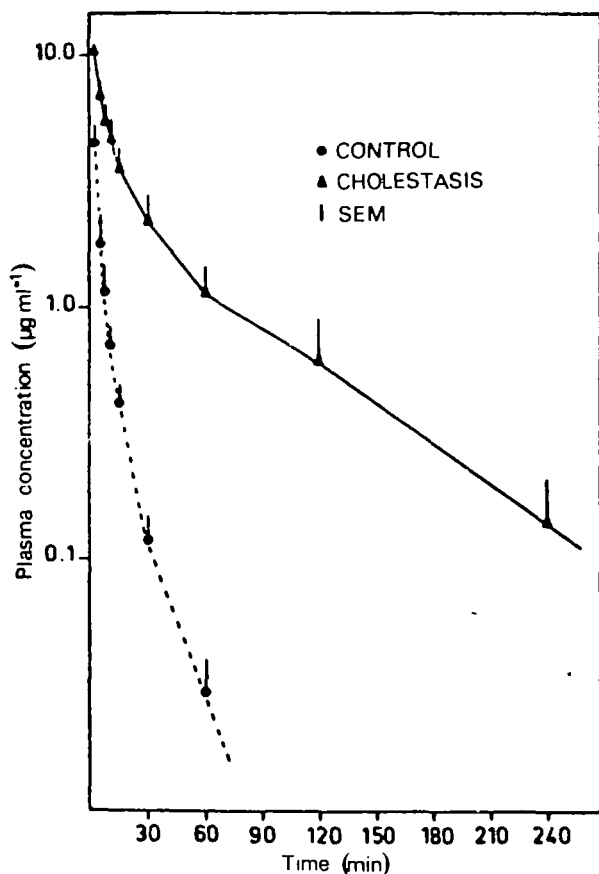


FIG. 4. Semilogarithmic plot of the mean plasma concentration-time profiles of Org 6368 in control cats ($n = 5$) and cats with cholestasis ($n = 5$).

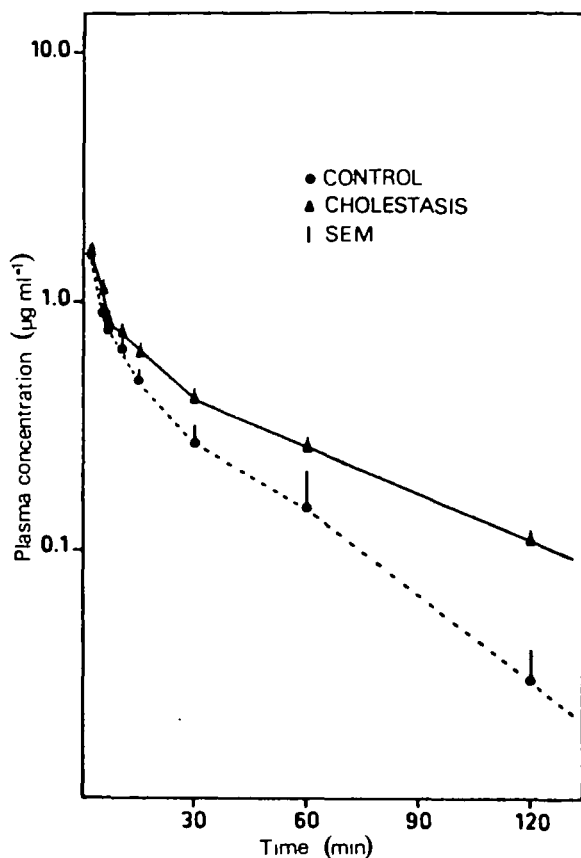


FIG. 6. Semilogarithmic plot of the mean plasma concentration-time profiles of pancuronium in control cats ($n = 5$) and cats with cholestasis ($n = 4$).

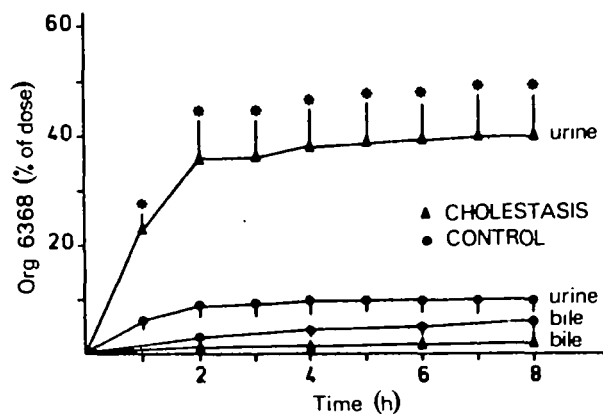


FIG. 5. Cumulative urinary and biliary excretion of Org 6368 and its metabolite in control cats ($n = 5$) and cats with cholestasis ($n = 5$). Mean \pm SEM. * Significant difference from control.

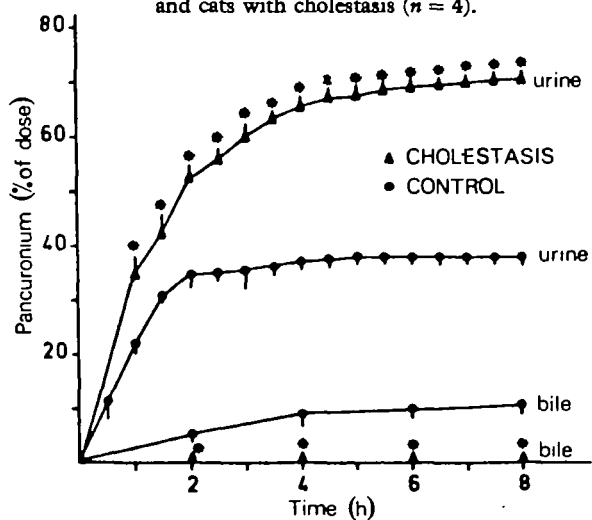


FIG. 7. Cumulative urinary and biliary excretion of pancuronium and its metabolites in control cats ($n = 5$) and cats with cholestasis ($n = 4$). Mean \pm SEM. * Significantly difference from controls.

administered dose, compared with the control cats. No statistically significant differences were found for the biliary excretion and the liver content after 8 h for Org 6368 between extrahepatic cholestasis and the control cats (fig. 5, table III). The total recovery of Org 6368 from bile, urine and liver in extrahepatic cholestasis was significantly greater than in the control cats (table III). There were no statistical differences in kinetics between the un-manipulated control cats and the sham-operated cats for Org 6368 (tables III, IV).

Pharmacokinetics of pancuronium

The elimination half-life $T_{1/2}$ for pancuronium in extrahepatic cholestasis differed from that in the control cats (table IV, fig. 6).

Cumulative urinary excretion of pancuronium showed a 1.8-fold increase compared with control (fig. 7).

Pancuronium could not be detected in the bile of cats with cholestasis (fig. 7), while the liver content of

pancuronium after 8 h was significantly less than in controls (table III). The total recovery from bile, urine and liver of pancuronium in extrahepatic cholestasis was significantly greater than in the control (table III).

Pharmacokinetics of gallamine

The pharmacokinetics for gallamine did not show significant differences between the group of cats with extrahepatic cholestasis and the control (tables III, IV, figs 8, 9).

Biotransformation

Thin-layer chromatography of urine, bile and liver samples of cats receiving Org 6368 was performed to obtain more information on biotransformation in extrahepatic cholestasis.

We found that, in extrahepatic cholestasis, Org 6368 is metabolized to its 3-hydroxy derivative to about the same extent as in control cats—approximately 60% of the administered dose after 8 h

TABLE III. The mean percentage of the dose of neuromuscular blocking drug in the liver, bile and urine 8 h after i.v. administration of pancuronium, Org 6368 and gallamine in control cats, sham-operated cats (only in case of Org 6368) and cats with cholestasis. * Figures in parentheses indicate the approximate percentage of the compounds which are present in liver, bile and urine in the form of metabolite(s). — = not detectable; n.d. = not determined.

Time (h)	Pancuronium		Org 6368			Gallamine	
	Control	Cholestasis	Control	Sham	Cholestasis	Control	Cholestasis
Liver 0-8	24.7 (—)*	13.1 (—)	55.0 (54.0)	56.4 (55.5)	49.0 (48.0)	5.2	11.3
Bile 0-2	5.1 (1.5)	0	3.1 (1.0)	n.d.	1.0 (0.5)	0.16	0.36
2-4	3.8 (1.0)	0	1.4 (0.6)	n.d.	0.4 (0.4)		
4-6	1.2 (0.9)	0	0.7 (0.4)	n.d.	0.5 (0.5)		
6-8	0.6 (0.6)	0	0.7 (0.3)	n.d.	0.4 (0.3)		
Urine 0-2	34.7 (0.2)	52.4 (3.5)	9.0 (1.0)	8.5 (2.3)	35.7 (7.9)	45.4	39.6
2-4	2.4 (0)	13.2 (3.8)	0.9 (0.3)	0.4 (0.1)	2.6 (2.6)	16.0	17.8
4-6	1.1 (—)	3.3 (—)	0.1 (0.1)	0.2 (0)	1.3 (1.2)	3.7	3.9
6-8	0	1.7 (—)	0.2 (0)	0.1 (0)	0.6 (0.6)	2.0	7.4

TABLE IV. Pharmacokinetic parameters after administration of pancuronium, Org 6368 and gallamine in control cats, sham-operated cats (only in the case of Org 6368) and cats with cholestasis. Mean \pm SEM. * Significant difference from control

	Pancuronium		Org 6368			Gallamine	
	Control	Cholestasis	Control	Sham	Cholestasis	Control	Cholestasis
$T_{1/2}^a$ (min)	4.98 \pm 1.37	9.04 \pm 5.35	2.46 \pm 0.32	1.47 \pm 0.14	5.91 \pm 1.61*	1.91 \pm 0.36	2.26 \pm 0.51
$T_{1/2}^b$ (min)	35.60 \pm 3.56	70.05 \pm 15.08*	13.64 \pm 0.97	8.69 \pm 1.96	47.46 \pm 10.58*	30.6 \pm 10.5	30.8 \pm 0.9
$T_{1/2}^c$ (min)						440 \pm 99	309 \pm 60
Cl (ml min ⁻¹ kg ⁻¹)	10.86 \pm 1.92	5.89 \pm 0.64*	51.30 \pm 10.76	78.73 \pm 28.14	8.15 \pm 3.55*	3.11 \pm 0.59	2.58 \pm 0.34
V_1 (litre kg ⁻¹)	0.174 \pm 0.042	0.187 \pm 0.027	0.263 \pm 0.073	0.233 \pm 0.063	0.123 \pm 0.012	0.097 \pm 0.012	0.117 \pm 0.020
V^{ss} (litre kg ⁻¹)	0.373 \pm 0.050	0.421 \pm 0.028	0.450 \pm 0.154	0.460 \pm 0.123	0.290 \pm 0.027	0.304 \pm 0.038	0.289 \pm 0.049

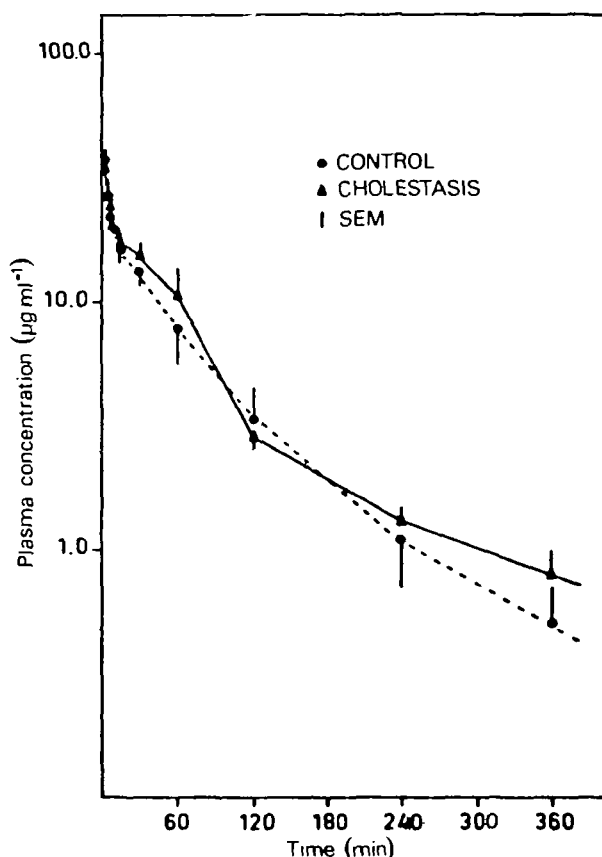


FIG. 8. Semilogarithmic plot of the mean concentration-time profiles of gallamine in control cats ($n = 5$) and cats with cholestasis ($n = 5$).

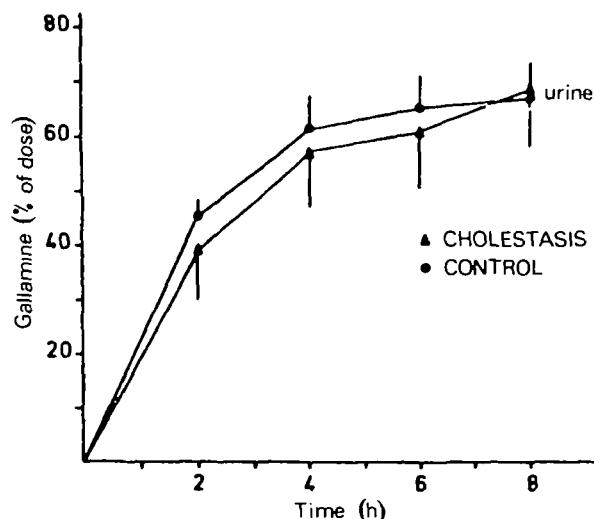


FIG. 9. Cumulative urinary excretion of gallamine in control cats ($n = 5$) and cats with cholestasis ($n = 5$). Mean \pm SEM.

(table III). At the end of the observation period, the livers in both groups contained virtually the 3-hydroxy derivative alone. The metabolism of pancuronium to its hydroxy-derivatives showed a pattern comparable to Org 6368 (table III).

The fluorimetric yields of livers after enzymatic pretreatment with β -glucuronidase and aryl-sulphatase did not differ significantly from that of non-enzyme treated livers.

DISCUSSION

The decrease in the plasma clearance and the concomitant prolongation of action of Org 6368 and pancuronium in cats with cholestasis may have several causes.

(a) *A decrease in renal clearance.* The physico-chemical properties of the bis(tris)-quaternary neuromuscular blocking drugs such as their relatively low lipid solubility and moderate protein binding (Meijer and Weitering, 1970; Waser, 1973; Meijer, Weitering and Vonk, 1976; Meijer et al., 1979) explain why renal elimination of such bis(tris)-quaternary drugs occurs by glomerular filtration (Cohen, Brewer and Smith, 1967; Raaflaub and Frey, 1972). However, renal failure as a cause of the marked decrease in the plasma clearance of the muscle relaxants Org 6368 and pancuronium in extrahepatic cholestasis observed in the present study is not very likely since renal function tests indicate a normal glomerular filtration rate. This conclusion is supported by the finding of normal kinetics of gallamine in the cats with cholestasis.

(b) *A decrease in metabolism.* Duvaldestin and colleagues (1978) investigated the fate of pancuronium in patients with liver cirrhosis and reported a decrease in the biotransformation of pancuronium to its 3-deacetylated metabolite. Possibly, plasma cholinesterase plays a role in the breakdown of the acetylated steroid compounds and this enzyme is synthesized by the liver (Kalow, 1959; Schuh, 1977). Biotransformation patterns of Org 6368 and pancuronium in cats with and without cholestasis in the present study were very similar. A similar result was found for pancuronium in man (Somogyi et al., 1977).

(c) *A decrease in biliary excretion.* The marked decrease in biliary output of pancuronium and Org 6368 in extrahepatic cholestasis cannot explain the pharmacological behaviour, since biliary excretion in control animals was small for both compounds. The biliary output of gallamine in extrahepatic cholestasis was not significantly different from control.

(d) *A decrease in hepatic uptake.* Inhibition of this process might explain the decreased plasma clearance of Org 6368 and pancuronium. The following observations point to a decreased hepatic uptake: the significant decrease in the liver content of pancuronium after 8 h in extrahepatic cholestasis, and the marked decrease in liver to plasma partition ratio for Org 6368.

The data and observations presented here support the concept that cholestasis causes an impairment of the hepatic transport at the uptake level. The mechanism of this effect could be an interaction of the steroid neuromuscular blocking drugs with naturally occurring bile components, most likely bile salts. Several authors reported impairment of biotransformation of drugs during extrahepatic cholestasis, caused by increased concentrations of bile salts (McLuen and Fouts, 1961; Hutterer et al., 1970). Vonk and others (1978a, b) observed that bile salts at concentrations of about 100 $\mu\text{mol litre}^{-1}$ and greater inhibit hepatocellular uptake of the organic cations tubocurarine and acetyl-procainamide ethobromide in intact rats, isolated rat livers and isolated hepatocytes. Recently, we have shown a prolongation of the duration of action of Org 6368 by bile salts as a result of inhibition of primary hepatic uptake (Vonk et al., 1979). The present investigations show that the concentrations of bile salt in plasma after 9–10 days cholestasis were increased from the normal value of 2 $\mu\text{mol litre}^{-1}$ to 500 $\mu\text{mol litre}^{-1}$. According to previous studies these increased concentrations could very well account for the impaired hepatic uptake of the bisquaternary steroidal agents.

There is no simple explanation for the greater total recovery of Org 6368 and pancuronium and their metabolite(s) in extrahepatic cholestasis. Formation of conjugates with sulphuric or glucuronic acid, or both, could be excluded. Similar findings were obtained (Buzello, 1975) for pancuronium in man. It is possible that changes in distribution in hepatic or extrahepatic tissues, or both, occurred.

The present results confirm the study of Somogyi, Shanks and Triggs (1977) that the use of pancuronium and other steroid neuromuscular blocking drugs in the commonly used dosage regime in patients with extrahepatic cholestasis may cause unexpected prolongation of action.

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EFFET D'UNE CHOLESTASE EXPERIMENTALE SUR LES AGENTS DE BLOCAGE NEUROMUSCULAIRE CHEZ LES CHATS

RESUME

On a comparé le pancuronium, Org 6368 et la gallamine sur des chats témoins et sur des chats ayant une cholestase expérimentale. On a constaté sur ces derniers une diminution du coefficient d'épuration plasmatique, ainsi qu'une prolongation du blocage neuromusculaire avec Org 6368 et le pancuronium; alors qu'aucune différence importante n'a été décelée dans le modèle de biotransformation d'Org 6368 et du pancuronium par rapport aux témoins. L'inhibition de la fixation hépatique d'Org 6368 et du pancuronium dans une cholestase extra-hépatique peut expliquer les modifications importantes survenues dans la pharmacocinétique des deux agents de blocage neuromusculaire stéroïdes. La pharmacocinétique de la gallamine a été normale pendant la cholestase. Les résultats laissent penser que dans des conditions pathologiques mettant en cause des concentrations plus fortes de sels biliaires dans le plasma, les agents de blocage neuromusculaire qui sont dégagés du plasma par le foie peuvent avoir une fixation hépatique altérée et donc une durée d'action prolongée.

EFFEKT VON EXPERIMENTELLER CHOLESTASE AUF NEUROMUSKULÄRE BLOCKIERUNGSDROGEN IN KATZEN

ZUSAMMENFASSUNG

Pancuronium, Org 6368 und Gallamin wurden verglichen— in einer Kontrollgruppe von Katzen, und bei Katzen mit experimenteller Cholestase. In letzterer Gruppe wurde eine sinkende Plasmaklärung und eine Verlängerung der neuromuskulären Blockierung bei Org 6368 und Pancuronium festgestellt; der Ablauf der Biotransformation dieser Drogen unterschied sich auch nicht von dem in der Kontrollgruppe. Die Unterdrückung der Leberaufnahme dieser beiden Drogen bei extrahepatischer Cholestase könnte die wesentlichen pharmakokinetischen Veränderungen der beiden Steroiddrogen erklären. Die pharmakokinetischen Vorgänge von Gallamin waren während Cholestase normal. Die Resultate zeigen, dass unter pathologischen Bedingungen—erhöhte Plasmakonzentrationen von Gallensalzen—neuromuskuläre Blockierungsmittel, die von der Leber aus dem Plasma geklärt werden, nicht so leicht von der Leber aufgenommen werden können und daher eine längere Wirkungsdauer haben können.

EFFECTO DE LAS DROGAS DE BLOQUEO NEUROMUSCULAR EN GATOS CON COLESTASIS EXPERIMENTAL

SUMARIO

Se compararon pancuronium, Org 6368 y gallamina en gatos de control y en gatos con colestasis experimental. En estos últimos se apreció, usando Org 6368 y pancuronium, una disminución en eliminación de plasma y una prolongación del bloqueo neuromuscular. No se detectó diferencia significativa alguna en el modelo de biotrans-

formación del Org 6368 ni en el del pancuronium, en comparación con el de los gatos de control. La inhibición de absorción hepática de Org 6368 y de pancuronium en colestasis extrahepática puede que explique la significativa alteración en las farmacocinéticas de las dos drogas esteroides de bloqueo neuromuscular. Las farmacocinéticas de la gallamina fueron normales durante la colestasis. Los

resultados sugieren que, bajo condiciones patológicas en las que acontezcan mayores concentraciones de sales biliares en el plasma, los agentes de bloqueo neuromuscular que el hígado extrae del plasma, pueden sufrir una absorción hepática perjudicial y, por consiguiente, una acción de duración prolongada.